J. DRUG DEL. SCI. TECH., 23 (5) 419-433 2013

Cell-mediated drug delivery to the brain

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The inability of most potent therapeutics to cross the blood-brain barrier following systemic administration necessitates the need to develop unconventional, clinically applicable drug delivery systems for the treatment of brain disorders. Smart, biologically active vehicles are crucial to accomplishing this challenging task. In this review, we discuss new drug delivery systems that utilize living cells for drug carriage to the brain. Using inflammatory response cells enables targeted drug transport and prolonged circulation times, along with reductions in cell and tissue toxicities. In addition, these cells are capable of cell-to-cell transmission of drug-laden nanoparticles that improves their therapeutic outcomes. Noteworthy, a proper differentiation of drug carriers into particular subtypes may further boost the therapeutic efficiency of cell-based drug formulations. Such systems for drug carriage and targeted release represent a novel strategy that can be applied to a spectrum of human disorders.

Key words: Brain – Cell-carriers – Drug delivery – Immunocytes – Nanoparticles – Targeted drug transport.

An extraordinary task, such as delivery of therapeutic agents to the brain, requires exceptional measures. Hidden behind the blood-brain barrier (BBB), and consequently inaccessible for the majority of substances circulating in the blood, the brain controls and regulates almost all critical processes within the organism [1]. Although small lipophilic molecules (MW < 400 kDa) can cross the BBB in pharmacologically significant amounts, effective concentrations of lipid-insoluble drugs (polar molecules and small ions), as well as high molecular weight compounds (peptides, proteins and nucleic acids), cannot be delivered to the central nervous system (CNS) within the limits of clinical toxicity. Brain microvessel endothelial cells (BMVEC) are important structural and functional components of the BBB that have tight extracellular junctions [2], relatively low pinocytic activity [3], and efflux protein systems (P-glycoprotein (Pgp), multidrug resistance proteins (MRPs) [4, 5], etc.) that pump compounds with the diverse structure back to the bloodstream. There is an additional enzymatic barrier for drugs with low stability that is maintained by the high density of mitochondria supplying the BMVEC with ATP necessary for enzymatic reactions. Furthermore, the activities of many enzymes participating in the metabolism of endogenous compounds, such as γ-glutamyl transpeptidase, alkaline phosphatase, and aromatic acid decarboxylase, are elevated in the cerebral microvessels [6]. Thus, the BBB represents an exceptional challenge for therapeutics designed to treat CNS disorders.

Immunocytes, mononuclear phagocytes (MP; monocytes, macrophages, and dendritic cells), lymphocytes, and neutrophils, as well as stem cells exhibit an intrinsic homing property enabling them to migrate to sites of injury, inflammation, and tumor across the BBB in response to the release of cytokines/chemokines and upregulation of certain cell surface proteins in the diseased tissues and nearby blood vessels. Even in the healthy brain, perivascular macrophages, which reside on the parenchymal side of endothelial cells, originate from circulating phagocytes, monocytes and macrophages. These cells have shown a remarkable capability to cross an intact BBB with 80 % turnover in 3 months [7, 8]. Many reports in the literature indicate that leukocytes traffic primarily between adjacent endothelial cells through the junctional complexes (paracellular migration) [9, 10]; or, in some cases, through the endothelial cell itself (transcellular migration) [11]. Under pathological conditions, the rate of immunocyte transport to the inflamed brain tissues is further elevated [12-18]. Such frequent migration offers a unique opportunity to design novel cell-based drug formulations. These drug delivery systems are based on living cells that act as "Trojan horses," carrying concealed drug cargoes across impermeable barriers (the BBB or blood-tumor barrier) to the disease sites. Importantly, immunocytes have a high rate of endocytosis that allows efficient accumulation of micro- and nanoparticles with incorporated drugs within intracytoplasmic endosomes, with subsequent release through the recycling processes that includes exocytosis. In addition, these cell-carriers have an extraordinary ability to reach metastases, and virtually inaccessible tissues (hypoxic areas within tumors with abrogated blood vessels). All these features make living cells attractive candidates for the CNS drug delivery. Two approaches can be utilized: i) loading cell-carriers with drugs that are usually incorporated into protective nanocontainers; or ii) genetic modification of the cells in order to produce therapeutically-active molecules. Using living cells as drug delivery vehicles takes advantage of their natural carriage, storage, mobility, and secretory capacities. It offers several benefits over common drug administration regimens. These include: targeted drug transport to disease sites, prolonged drug half-lives, time-controlled drug release, and diminished drug immunogenicity and cytoxicity profiles. In the following chapters we will discuss the benefits and hurdles for this methodology.

Different approaches may be applied in clinical settings (*Figure 1*). First, cell-carriers may be harvested from peripheral blood by apheresis, then genetically modified or loaded with drug-incorporated particles, and re-infused into the patient (Approach I). An alternative approach may be harvesting stem cells from bone marrow, propagating them in culture to obtain specific cell types or even subtypes, and then transfecting/transducing the cells or loading them with a drug nanoformulation (Approach II). This method will allow for the expansion of the cell population, and their propagation with definable properties, although this would require a more invasive procedure [19]. Finally, drug loading into cell-carriers can be achieved directly in the patient's peripheral blood, when nanoformulated therapeutic agents are injected and selectively taken up by circulating cell-carriers (Approach III). Targeting of such nanocarriers could be achieved by coating the surface of nanocarriers with the receptor-specific moieties to: i) mannose receptor that is expressed on macrophages/monocytes and dendritic cells (by mannose [20-22] or DEC-205 antibodies) [23]; ii) integrins on monocytes/macrophages and neutrophils (by RGD peptide, gelatin, fibronectin, and collagen) [24]; iii) folate receptor on pro-inflammatory M1 macrophages and tumor associated macrophages (TAMs, by

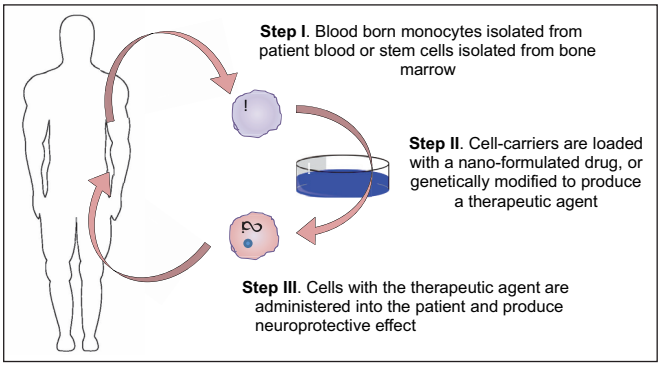

Figure 1 - Cell-mediated drug delivery systems in clinical settings. Three approaches for cell-mediated drug delivery systems in clinical settings. Approach I. Immune cells harvested from peripheral blood by apheresis. Approach II. Stem cells harvested from patient's bone marrow propagated in culture. Then, collected cells are loaded with drug nanoformulations or genetically-modified to produce therapeutic proteins, and re-infused into the patient. Approach III. A drug-encoding pDNA or a therapeutic agent itself are formulated into protective nanocontainer/complex and targeted to the cell-carriers. Then, the vectorized drug nanoformulation is systemically administered into the patient.

folate molecules) [25]; iv) scavenger receptor in macrophages and dendritic cells (by negative charge of nanocarriers' surface) [26, 27]; v) complement receptor on immune cells (by opsonization process), or/and vi) Fc receptors on monocytes/macrophages (by Fc portion of antibodies) [28]. In some cases, specific receptors with Fab regions may be targeted by antibodies, for example, CD3 receptor on T-cells [29], or CD11b/CD18 receptors on monocytes/macrophages. Indeed, the complexity of these interventions is challenging, yet they promise an unparalleled efficacy in the treatment of many life-threatening conditions including those lacking the effective pharmacotherapy. In this review we will first, discuss unique mechanisms that the cell-carriers may offer for drug transport, and then, give specific examples of this approach in different disease models (*Table I*).

I. TARGETING CELL-CARRIERS TO THE BRAIN ("TROJAN HORSE" EFFECT)

Drug targeting to sites of tissue injury, tumor or infection with limited toxicities is one of the main goals for successful pharmaceutics. Immune cells are highly mobile, capable of travel toward inflammation signals of cells with the ability to cross the endothelial wall due to their increased margination and extravasation [1]. Indeed, the numbers of cell-carriers that can penetrate the BBB and reach the disease site is crucial for the therapeutic efficacy of cell-mediated drug formulations. Inflammation is a common denominator for almost every neurodegenerative disorder. These include Alzheimer's and Parkinson's diseases (AD and PD), stroke, traumatic brain injury (TBI), multiple sclerosis (MS), age-related macular degeneration (AMD), prion disease, meningitis, encephalitis and human immunodeficiency virus (HIV)-associated neurocognitive disorders, epilepsy, brain cancer, and even mental disorders such as depression, autism, and schizophrenia. The disease state is accompanied by extensive immunocytes recruitment [18]. At these conditions, the cells migrate toward the inflammation site via the processes known as diapedesis and chemataxis [30], and cross the BBB causing the barrier breakdown [13, 14, 17, 31]. Interestingly, blood circulating monocytes are capable of crossing the BBB and then differentiating into microglia at the sites of neurodegeneration [32, 33]. Reactive oxygen species (ROS) produced in many pathological disorders further increase BBB permeability [34]. Thus, many studies have reported the migration of inflammatory-response cells to the brain with HIV-related dementia [12, 16], AD [35, 36], cerebral ischemia [15], and injury sites such as infarcted myocardium [37, 38], brain and spinal cord injury [39-42]. Hence, the inflammation process at the disease site provides the opportunity for active targeted drug delivery using inflammatory-response cells as vehicles.

The first reports suggesting that living cells may have a therapeutic potential for targeted drug delivery across biological barriers were published in 1980s [43, 44]. In particular, transport of peripheral blood neutrophils (PMNs) loaded with fluorescently radioactively-labeled liposomes was studied across confluent Madin Darby canine kidney (MDCK) epithelial cell monolayers *in vitro* [44]. Transmission electron micrographs demonstrated that, in response to the chemotactic signal, PMNs adhered to the apical surface of MDCK cells, emigrated across the MDCK cell layer, passed through the 3-micron pores in the polycarbonate membrane, and finally, appeared in the bottom well. Noteworthy, most, if not all, of the migrated PMNs contained a fluorescent dye, Lucifer yellow, that was used to stain liposomes, suggesting these cells may be used for the transport of loaded, nanoformulated therapeutics across biological barriers.

Since then, different studies have demonstrated the successful cell-mediated delivery of therapeutics to inflamed brain tissues [19, 45-51]. Typically, to study cell-based drug formulations, bone marrow-derived progenitor cells were isolated from donor animals, differentiated in culture to produce a specific type of carrier cells, loaded or genetically modified to produce a therapeutic agent, and adoptively transferred to the animal with a model disease (*Figure 2*). For example, a potent anti-inflammatory polypeptide, catalase, was delivered to the brain in therapeutically significant amounts by bone-marrow-derived monocytes (BMM) in PD murine models induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [45] or 6-hydroxidophamine (6-OHDA) intoxications [46]. To preclude the polypeptide degradation inside the cell-carriers, catalase was incorporated into a polyion complex micelle ("nanozyme") with a synthetic polyelectrolyte block copolymer. Approximately 2.1 and 3.4 % of the injected dose of systemically administered catalase was delivered by the BMM to the brain with MPTP- and 6-OHDA-triggered inflammation, respectively. Indeed, in healthy mice without brain inflammation, a majority of systemically administered macrophages became trapped in peripheral organs such as lungs, liver, and spleen, and then cleared out [52]. Nevertheless, when brain inflammation developed, systemically administered BMM targeted the disease tissues. Thus, catalase was detected over 16-20 days after the cells intravenous (i.v.) administration in the inflamed brain of mice intoxicated with lipopolysaccharides (LPS) (*Figure 3A*), and only for 1-2 days in healthy control animals (*Figure 3B*) [46]. Interestingly, on the second week after the BMM adoptive transfer in LPS-intoxicated mice, the main distribution of

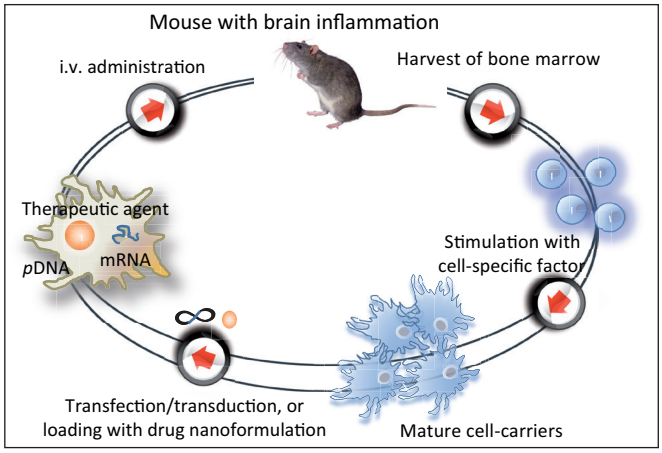

Figure 2 - A pictorial scheme for cell-based drug delivery evaluations. Bone marrow-derived progenitor cells are isolated from donor animals, differentiated in culture to produce specific type/subtype of carrier cells, loaded or genetically modified to produce a therapeutic agent, and re-administered to animals with brain inflammation for gene and protein delivery.